

Claims

1. A method for screening a first repertoire of molecules against a second repertoire of molecules to identify those members of the first repertoire which interact with members of the second repertoire, comprising :

- (b) arranging the first and second repertoires to form at least one array, such that all members of the first repertoire are juxtaposed to all members of the second repertoire; and
- (b) detecting an interaction between the members of the first and second repertoires, thereby identifying those members of the first repertoire that interact with members of the second repertoire.

2. The method according to claim 1, wherein members of both the first repertoire and the second repertoire are arranged in a series of lines, channels or tubes, each containing a member of the first or second repertoires such that the lines, channels or tubes corresponding to the first repertoire and those corresponding to the second repertoire are juxtaposed to one another so that all members of the first repertoire are juxtaposed with all members of the second repertoire.

3. The method according to claim 2, wherein each line, channel or tube comprises a group of different members of the first and second repertoires, respectively.

4. The method according to any one of claims 1 to 3 wherein members of the first and second repertoires are run along channels cut or etched into a solid material such that all the channels containing members of the first repertoire intersect all the channels containing members of the second repertoire.

5. The method according to any one of claims 1 to 3, wherein members of the first and second repertoires are applied to a single support.

6. The method according to any one of claims 1 to 3, comprising the steps of:

- (a) arranging the first and second repertoires on first and second supports;
- (b) juxtaposing the first and second supports such that all members of the first repertoire are juxtaposed with all members of the second repertoire; and
- (c) detecting an interaction between the members of the first and second repertoires.

7. The method according to claim 1, wherein the first and second repertoires are selected from the group consisting of repertoires of: peptides; polypeptides; nucleic acid molecules;

purified proteins; recombinant proteins; amino acids; cDNAs; expressed cDNAs; oligonucleotides; nucleotides; nucleotide analogues; families of related genes or the corresponding proteins thereof; enzymes; DNA binding proteins; immunoglobulin family members; antibodies; T cell receptors; haptens; small organic molecules; non-organic compounds; metal ions; and carbohydrates.

8. The method according to claim 1, wherein the interaction between the members of the first and second repertoires is selected from the group consisting of: a binding interaction; DNA methylation; nucleic acid degradation; nucleic acid cleavage (single or double stranded); a signalling event; a catalytic reaction; a phosphorylation event; a glycosylation event; proteolytic cleavage; a chemical reaction; and cellular infection.

9. A method for screening first, second and third repertoires of molecules against each other to identify those members of the first, second and third repertoires which interact, comprising :

- (a) arranging the first, second and third repertoires to form at least one array, such that all members of the first, second and third repertoires are juxtaposed; and
- (b) detecting an interaction between the members of the first, second and third repertoires, thereby identifying those members of the first, second and third repertoires that interact.

10. A method for creating a combinatorial library of two-chain polypeptides, each member of which library comprises one member of a first repertoire of single chain polypeptides and one member of a second repertoire of single chain polypeptides, which method comprises the step of arranging the first and second repertoires of single chain polypeptides, such that all members of the first repertoire intersect all members of the second repertoire, thereby generating at their intersections all combinations of functional two-chain polypeptides, thereby creating a combinatorial library of two-chain polypeptides.

11. A method of screening the combinatorial library of two-chain polypeptides of claim 10 for binding to a target molecule, the method comprising the step of detecting the interaction between the two chain polypeptides and the target molecule.

12. The screening method according to claim 11, wherein the combinatorial library is screened for interactions with more than one target molecule.

13. A method for creating a combinatorial library of three-chain polypeptides, each member of which library comprises one member of a first repertoire of single chain

polypeptides, one member of a second repertoire of single chain polypeptides, and one member of a third repertoire of single chain polypeptides, which method comprises:

(a) arranging the first, second and third repertoires of single chain polypeptides, such that all members of each repertoire intersect all members of the other two repertoires, thereby generating at their intersections all combinations of functional three-chain polypeptides, thereby creating a combinatorial library of three-chain polypeptides.

14. A method of screening the combinatorial library of three-chain polypeptides of claim 13 for binding to a target molecule, the method comprising the step of detecting the interaction between the three chain polypeptides and the target molecule.

15. The screening method according to claim 14, wherein the combinatorial library is screened for interactions with more than one target molecule.

16. The method according to any one of claims 1, 9, 11, 12, 14 or 15, whereby the pattern of interactions between the first, second, and, if present, third, repertoires identifies positive interactions, negative interactions, specific interactions or cross-reactive interactions, or whereby the pattern of interactions is used to construct a phylogenic tree inferring the similarity between members of the first repertoire according to the pattern of interactions with the second and/or, if present, the third repertoire; between members of the second repertoire, according to the pattern of interactions with the first and/or, if present, the third repertoire; and/or between members of the third repertoire, according to the pattern of interactions with members of the first and/or the second repertoire.

17. The method according to claim 1 or claim 9, whereby one or more of the first, second and, if present, third repertoires comprises a plurality of nucleic acid molecules which are expressed to produce their corresponding polypeptides *in situ* in the array.

18. The method according to claim 17, wherein the nucleic acid molecules are in the form of expression vectors which encode polypeptide members of the repertoire, operatively linked to control sequences sufficient to direct the transcription of the nucleic acid molecules.

19. The method according to claim 18, wherein the expression vector is a bacteriophage.

20. The method according to claim 19, wherein the expression vector is a plasmid.

21. The method according to claim 19, wherein the expression vector is a linear nucleic acid molecule.

22. The method according to any one of claims 17 to 21, wherein the nucleic acids are contained and expressed within cells.

23. The method according to claim 22, wherein the cells are selected from the group consisting of bacterial cells, lower eukaryotic cells and higher eukaryotic cells.

24. The method according to any one of claims 17 to 21, wherein the nucleic acid molecules are immobilised in the form of naked or complexed nucleic acid.

25. The method according to claim 1 or claim 9, wherein the members of at least one repertoire are arrayed using robotic means.

26. A method for screening a first repertoire of molecules against a second repertoire of molecules to identify those members of the first repertoire which do not interact with members of the second repertoire, the method comprising:

- (a) arranging the first and second repertoires, such that all members of the first repertoire are juxtaposed with all members of the second repertoire; and
- (b) identifying those members of the first and second repertoires that do not interact with one another.

27. A method for screening a first repertoire of molecules against a second repertoire of molecules to identify members of the first and second repertoires whose interactions with one another are dependent on the presence or absence of a third molecule or set of molecules, comprising:

- (a) arranging the first and second repertoires, such that all members of the first repertoire are juxtaposed with all members of the second repertoire; and
- (b) detecting interactions between members of the first repertoire and members of the second repertoire in the presence of different concentrations of the third molecule or set of molecules, such that members of the first and second repertoires whose interactions with one another are dependent on the presence or absence of the third molecule or set of molecules are identified.

28. The method according to claim 27, wherein the interaction of the third molecule or set of molecules with one or more members of the first repertoire enables such members of the first repertoire to interact one or more members of the second repertoire.

29. The method according to claim 27, wherein the interactions between the members of the first and second repertoire require the simultaneous binding of these members to the third molecule or set of molecules.

30. The method according to claim 27, wherein the interactions between the members of the first and second repertoire are enhanced by the presence of a third molecule or set of molecules.

31. The method according to claim 27, wherein the interactions between the members of the first and second repertoire are blocked by the presence of a third molecule or set of molecules.

32. An apparatus for drawing, on a medium, lines of matter comprising members of a first, second and, optionally third repertoire of molecules.

33. An apparatus which comprises intersecting channels or tubes along which members of a first, second and, optionally third repertoire of molecules can pass.

34. The method according to claim 1, wherein the first and second repertoires are dispensed to form the array, and wherein fewer dispensing events are required than the number of interactions to be tested.

35. The method according to claim 34, wherein members of at least one, but not all, repertoires are arranged in a series of lines, channels or tubes, each containing a member of that repertoire such that the lines, channels or tubes corresponding to that repertoire intersect with all members of the other repertoires.

36. The method according to claim 34 or claim 35 wherein members of both the first repertoire and the second repertoire are dispensed into a series of lines, channels or tubes, each containing a member of the first or second repertoires such that the lines, channels or tubes corresponding to the first repertoire and those corresponding to the second repertoire contact one another so that more interactions are tested than dispensing events are required.

37. A method for optimizing conditions for a biological interaction, which method comprises creating all combinations of two or more different sets of variable parameters at the intersections of two or more different sets of intersecting lines, channels or tubes, and assaying the biological interaction, thereby optimizing the conditions for the biological interaction.

38. A method according to claim 37, wherein the variable parameters are selected from the group consisting of: a buffer composition, a substrate concentration, pH, temperature, the presence of denaturants and the presence of renaturants.

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39. A method for screening a first and a second repertoire of enzymes to identify those members of the first repertoire and those members of the second repertoire which together participate in a two or more step enzymatic reaction that creates a given product from a given substrate, which method comprises:

- 10 (a) arranging the first and second repertoires to form at least one array, such that all members of the first repertoire are juxtaposed to all members of the second repertoire; and
- (b) detecting the formation of the given product at the intersections of the members of the first and second repertoires, thereby identifying those members of the first and second repertoires which together participate in a two or more step enzymatic reaction that creates the given product from the given substrate.
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40. A method for screening a plurality of cellular populations against a plurality of viral populations to identify those viral populations among the plurality of viral populations that infect cellular populations among the plurality of cellular populations, which method comprises:

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- (a) arranging the plurality of cellular populations and the plurality of viral populations to form at least one array, such that all the different cellular populations are juxtaposed with all the viral populations; and
- 25 (b) detecting viral infection in the plurality of cellular populations, thereby identifying those viral populations among the plurality of viral populations that infect cellular populations among the plurality of cellular populations.

30 41. A method for screening a plurality of different cellular fractions against one another to identify those cellular fractions that contain components which interact with components in the other cellular fractions, which method comprises:

- (a) arranging the plurality of cellular fractions to form at least one array, such that all the different cellular fractions are juxtaposed to one another; and
- 35 (b) detecting the interaction of different cellular fractions at sites where the different cellular fractions are juxtaposed, thereby identifying those cellular fractions that contain components which interact with components in the other cellular fractions tested.

42. A method for screening a plurality of different cellular populations against one another to identify those cellular populations that interact with the other cellular populations, which method comprises:

- (a) arranging the plurality of cellular different populations to form at least one array, such that all the different cellular populations are juxtaposed to one another; and
- (b) detecting the interaction of different cellular fractions at sites where the different cellular fractions are juxtaposed, thereby identifying those cellular populations that interact with the other cellular populations tested.

43. A method for screening a peptide repertoire against the same peptide repertoire to identify those members of the peptide repertoire that interact with other members of the peptide repertoire, which method comprises:

- (a) arranging the members of the peptide repertoire to form at least one array, such that all the members of the peptide repertoire are juxtaposed to one another; and
- (b) detecting the interaction of different members of the peptide repertoire at sites where the different members are juxtaposed, whereby those members of the peptide repertoire that interact with other members of the same peptide repertoire are identified.

44. A method for screening a polypeptide repertoire against the same polypeptide repertoire, in order to identify those members of the polypeptide repertoire that interact with other members of the polypeptide repertoire, which method comprises:

- (a) arranging the members of the polypeptide repertoire to form at least one array, such that all the members of the polypeptide repertoire are juxtaposed to one another; and
- (b) detecting the interaction of different members of the polypeptide repertoire at sites where the different members are juxtaposed, thereby identifying those members of the polypeptide repertoire that interact with other members of the polypeptide repertoire.

45. The method according to any one of claims 1, 43 or 44, which method uses the yeast two-hybrid system to identify those members of the repertoires of molecules that interact with one another.

46. A method according to any one of claims 39 to 44, which method uses a series of intersecting lines, channels or tubes to create the array.

47. The method of claim 45, which method uses a series of intersecting lines, channels or tubes to create the array.

48. A method for creating a combinatorial library consisting of all members of a first repertoire of polypeptides paired with all members of a second repertoire of polypeptides, which method comprises:

- (a) arranging a repertoire of host cells containing a plurality of nucleotide sequences encoding a first repertoire of polypeptide members, and a plurality of nucleotide sequences encoding a second repertoire of polypeptide members to create an array, such that cells containing nucleotide sequences encoding all members of the first repertoire intersect with nucleotide sequences corresponding to all members of the second repertoire; and
- (b) transforming the cells containing the nucleotide members of the first repertoire with the nucleotide sequences that encode the members of the second repertoire where the two repertoires intersect; and
- (c) expressing the nucleotide sequences to produce the corresponding polypeptides of the first and second repertoires; thereby creating a combinatorial library consisting of all members of the first repertoire of polypeptides paired with all members of the second repertoire of polypeptides.

49. A method for screening the combinatorial library created according to claim 48 for members of the first repertoire that interact with members of the second repertoire, the method comprising the step of detecting an interaction between the polypeptide members of the first and second repertoires, thereby identifying members of the first repertoire that interact with members of the second repertoire.

50. A method for creating a combinatorial library consisting of all members of a first repertoire of polypeptides paired with all members of a second repertoire of polypeptides, which method comprises:

- (a) arranging a repertoire of host cells containing a plurality of nucleotide sequences encoding a first repertoire of polypeptide members, and a plurality of viruses containing a plurality of nucleotide sequences encoding a second repertoire of polypeptide members to create an array, such that cells containing all nucleotide members of the first repertoire intersect with viruses containing all nucleotide members of the second repertoire;
- (b) infecting the cells containing the nucleotide members of the first repertoire with the viruses that contain the nucleotide members of the second repertoire where the two repertoires intersect; and
- (c) expressing the nucleotide sequences to produce the corresponding polypeptides of the first and second repertoires, thereby creating a combinatorial library consisting of all

members of the first repertoire of polypeptides paired with all members of the second repertoire of polypeptides.

51. A method of screening the combinatorial library created according to the method of claim 50 to identify members of the first repertoire that interact with members of the second repertoire, said method comprising the step of detecting an interaction between polypeptide members of the first and second repertoires, whereby members of the first repertoire that interact with members of the second repertoire are identified.

52. A method for creating a yeast two hybrid library consisting of all members of a first repertoire of polypeptides paired with all members of a second repertoire of polypeptides, which method comprises:

- (a) arranging yeast cells containing a plurality of nucleotide sequences encoding a first repertoire of polypeptide members, and yeast cells containing a plurality of nucleotide sequences encoding a second repertoire of polypeptide members to create an array, such that yeast cells containing all nucleotide members of the first repertoire intersect with yeast cells containing all nucleotide members of the second repertoire;
- (b) allowing the yeast cells containing the members of the first repertoire to mate with the yeast cells containing the members of the second repertoire where the two repertoires intersect; and
- (c) expressing the nucleotide sequences to produce the corresponding polypeptides of the first and second repertoires, thereby creating a yeast two hybrid library consisting of all members of a first repertoire of polypeptides paired with all members of a second repertoire of polypeptides.

53. A method of screening a combinatorial library created according to the method of claim 52 to identify members of the first repertoire that interact with members of the second repertoire, the method comprising the step of detecting an interaction between the polypeptide members of the first and second repertoires, whereby members of the first repertoire that interact with members of the second repertoire are identified.

54. The method according to any one of claims 48, 50, or 52, which method uses a series of intersecting lines, channels or tubes to create the array.

55. A method according to any one of claims 1, 9, 10, 13, 26, 27, 37, 39-44, 48, 50, or 52 whereby the members of the repertoires are directed to their positions in the array by means of a tagging system, such that a particular member of the repertoire binds a line, channel or tube.